Eur J Vasc Endovasc Surg **24**, 105–116 (2002)
doi:10.1053/ejvs.2002.1692, available online at http://www.idealibrary.com on IDEAL®

REVIEW ARTICLE

Familial Abdominal Aortic Aneurysm: a Systematic Review of a Genetic Background

C. J. van Vlijmen-van Keulen*[1], G. Pals[2] and J. A. Rauwerda[1]

[1]*Institute for Cardiovascular Research and* [2]*Department of Clinical Genetics, Vrije Universiteit Medical Center, Amsterdam, The Netherlands*

Background: *familial clustering of the abdominal aortic aneurysm (AAA) is clear, 12–19% of AAA patients have one or more first-degree relatives with an aneurysm and 4–19% is detected with ultrasound screening.*
Objectives: *to review the genetic background of AAA.*
Design, methods and materials: *computer searches of the MEDLINE, EMBASE, SUMsearch database and the Cochrane Library and searched reference lists of English language articles concerning the genetics of AAA, candidate gene approach and linkage analysis.*
Results: *brothers of AAA patients are at high risk to develop an AAA. The candidate gene approach was performed to detect defects in one of the components of the connective tissue, i.e. type I and III collagen, elastin and fibrillin, the inflammatory cell-derived matrix metalloproteinase, there inhibitors, auto-immune components and components related to atherosclerosis.*
Conclusion: *these studies give us insight in the pathology but do not lead to the specific genetic factor(s) responsible for (familial) AAA. Considering the supposed autosomal dominant inheritance, a gene mutation in one of the structural proteins of the connective tissue is expected. In the future, linkage analysis may resolve the genetic background of AAA.*

Key Words: Familial abdominal aortic aneurysm; Genetic aetiology; Candidate genes; Linkage analysis.

Introduction

Despite improved diagnostic and therapeutic options, patients with an aneurysm of the abdominal aorta (AAA) still have an increased risk of mortality. In screening programs the prevalence of an AAA, defined by an infrarenal aortic diameter $\geq$30 mm based on using ultrasound imaging, varied between 4.5 and 8% in men aged between 60 and 80 years.[1,2] The incidence of new aneurysms, defined as an initial aortic diameter smaller than 30 mm that has expanded more than 5 mm in a 5.5 year period, is 3.5 per 1000 person-years (95% confidence interval (CI) 2.8–4.4). The highest incidence, of new diagnosed aneurysms, i.e. 5.2 (95% CI 3.7%–7.0), was found in men aged from 60–69 years.[3]

The aetiology of the AAA is assumed to be multifactorial. Positive associations with AAA include age,[4–7] smoking,[7–13] and family history of AAA[14–24], whereas atherosclerotic disease, female sex, diabetes and black race are negatively associated.[9] Clifton, who described familial AAA that affected three brothers of one family, was the first to hypothesise that AAA could be an inheritable disease.[14]

The purpose of this review is to give an overview of research data on the genetic background of AAA. Studies were evaluated that investigated familial AAA by using family history or ultrasound examination. The outcome is discussed in relation with present knowledge on the inheritance of AAA, the candidate gene approach, and linkage analysis.

Methods

Using the words or MeSH terms aortic aneurysm (abdominal), screening, aetiology, genetics (linkage), extracellular matrix proteins, matrix metalloproteinases and tissue inhibitors of metalloproteinases, we performed a computerised search for relevant

* Please address all correspondence to: C. J. van Vlijmen-van Keulen, Department of Vascular Surgery, PO Box 7057, 1007 MB Amsterdam, The Netherlands.

1078–5884/02/020105 + 12 $35.00/0

articles in MEDLINE, EMBASE, SUMsearch electronic databases and the Cochrane library. We also searched reference lists of review and original articles. We limited our search to English language articles. We included all available material untill April 2002.

We performed a systematic review for the subheading "Family history and Ultrasound screening". Studies were included in the analysis if they had used the following definitions:

- a positive family history, defined as one or more first-degree relatives (FDR) with an AAA mentioned by the patient and/or diagnosed by a physician;
- ultrasonography for AAA, defined as an infrarenal aortic diameter (IRD) greater than 30 mm, or a diameter of 50% greater than the normal suprarenal aorta (SRD);
- studies had to compare the patient group with a control group.

We calculated the odds ratios with corresponding 95% confidence intervals (95% CI).

Our findings are discussed in relation with current knowledge on inheritance, candidate gene approach and linkage analysis.

Results

Family history and ultrasound screening

In this systematic review, 29 candidate studies were evaluated that investigated familial occurrence of AAA by family history or ultrasound examination, compared to a control group. Four articles fulfilled our criteria (Table 1). Figure 1 shows that the odds ratio for an AAA in first degree relatives mentioned by the AAA patient is 9.7, with the 95% CI from 4 to 23.[16,18] The odds ratio for siblings of AAA patients is 4.1 (95% CI 1.5–11.2) and for the brothers 4.2 (95% CI 1.4–12.8).[16,18,21]

Using infrarenal aortic diameter based on ultrasound measurements, the siblings of AAA patients have an odds ratio between 2.6 (95% CI 1.0–7.1) and 3.3 (95% CI 1.1–10.7).[21,25] In the study of Baird *et al*.[21] ultrasound examinations were done only for siblings of the AAA patients. Controls themselves were examined, but not their siblings. In the study of Salo *et al*. we calculated an odds ratio for brothers of AAA patients of 8.1 (95% CI 1.8–37.5).[25]

Because these studies were of a cross-sectional design, it is always uncertain whether any other relative will develop an AAA in the future.

The evaluation of familial occurrence of AAA showed that AAA in siblings is significantly higher than in the overall population, especially among the male population of the same age, supporting that AAA can be an inheritable disease.

Inheritance

Different models have been postulated regarding the pattern of inheritance of AAA. Tilson and Seashore studied the hereditary pattern of 50 families, among these three pairs of identical twins. They suggested that only one gene is responsible for AAA inheritance which is likely to be autosomal dominant with a strong bias toward male to female expression (8 : 1). An X-linked inheritance pattern was ruled out based on transmission of the disease from father to son in four of the families included.[26] Powell and Greenhalgh on the other hand assumed a multifactorial model and calculated a 70% inheritability from 60 patients, 25 with a positive family history, using the threshold model for inheritability by Falconer.[17,27] Majumber *et al*. collected data on first-degree relatives of 91 probands. Thirteen families had at least one affected FDR, one family had a proband with an affected spouse and the remaining families were simplex. The most likely genetic model was a recessive gene at an autosomal diallelic major locus.[28] Verloes *et al*. assumed a single gene effect showing dominant inheritance, with low penetrance.[29] The latter two authors noted that a multifactorial component did not increase the likelihood of the data set significantly. Data on family history for AAA collected in our previous study were in agreement with an autosomal dominant inheritance with reduced penetrance.[30]

In all these family studies that investigated the inheritance pattern of AAA no classic signs of any known syndrome familiar with AAA, like the Marfan or Ehlers Danlos type IV syndrome, could explain the heredity pattern.

Since familial AAA may occur in two or three consecutive generations autosomal dominant is the most likely pattern of inheritance.

Candidate genes

The candidate gene approach involves the characterisation of mutations in the candidate gene. Possible candidate genes may be genes encoding the components of the extracellular matrix of the vascular wall. This matrix mainly consists of collagen. Other candidate proteins are elastin, proteoglycans, fibrillin

Table 1. Attribute of included studies with search terms family history and ultrasonography.

Author	Year	Population	Design	Control group	Results of positive family history*(FH)	Results of ultrasonography (US)#	Comment
Johansen *et al.*[16]	1986	250 AAA patients, mean age 72 year (±10.6) answered questionnaire	Retrospective study	250 non aneurysm patients, mean age 57.2 year (±17.2) answered questionnaire	48 (40♂, 8♀, 19.2%) of AAA patients compared to 6 (5♂, 1♀) of 250 (2.4%) of the control group		The relative risk for AAA among persons with an affected FDR was 11.6
Darling *et al.*[18]	1989	542 consecutive AAA patients coming up for surgery	9 year Prospective study	500 non aneurysm patients, similar age and sex distribution	82 (15.1%) AAA patients compared to 9 (1.8%) of the control group		
Baird *et al.*[21]	1995	427 siblings of 126 AAA patients, age over 50	Retrospective, cross-sectional study	451 siblings of 100 cataract patients	19 (4.4%, mean age 67.7±8.8) siblings compared to 5 (1.1%) siblings of the controls had probably¶ or definite AAA	54 siblings underwent US, 10 (19%) had an AAA compared to 8 (8%) of the control group	The risk of AAA began at an earlier age and increased more rapidly for siblings of AAA probands than for siblings of controls or controls themselves
Salo *et al.*[25]	1999	241 FDR, >50 year, of 112 AAA probands	Retrospective, cross-sectional study	284 controls, similar age and sex distribution		11 (4.6%) FDR had an AAA, compared to 4 (1.4%) in the control group	Of men over 60, 18% and in men over 65, 22% had AAA, compared to 2.6% and 3.1%, respectively, in the control group

* A positive family history (FH) is defined as one or more first-degree relative (FDR) with an AAA mentioned by the patient and/or diagnosed by a physician.
# AAA by ultrasonography (US) is defined as an infrarenal aortic diameter (IRD) greater than 30 mm, or a diameter of 50% greater than the normal suprarenal aorta (SRD).
¶ Probably AAA was defined, as there was sufficiently descriptive data to meet the criteria for AAA but medical records could not be obtained.
FDR means first-degree relative.

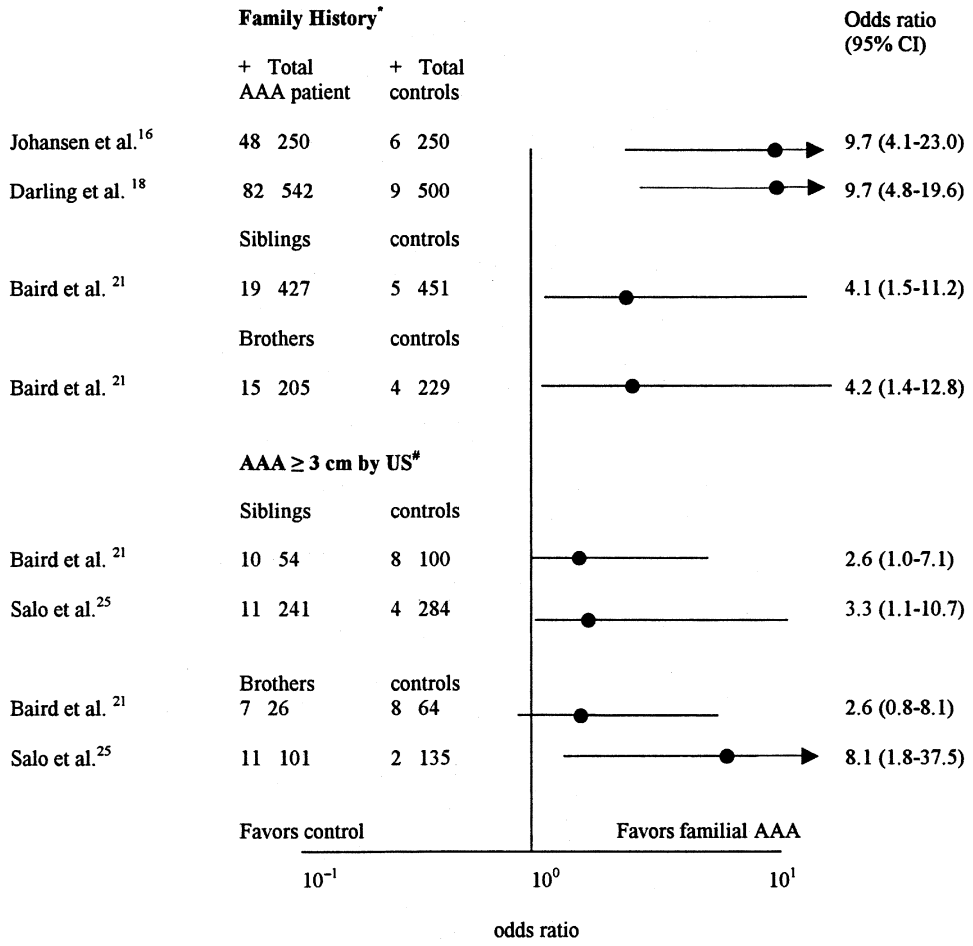

Fig. 1 Odds ratios of a positive family history and/or ultrasound examination for AAA in individual studies. * A positive family history (FH))is defined as one or more first-degree relative (FDR) with an AAA mentioned by the patient and/or diagnosed by a physician. # AAA by ultrasonography (US) is defined as an infrarenal aortic diameter (IRD) greater than 30 mm, or a diameter of 50% greater than the normal suprarenal aorta (SRD).
Siblings are the brothers and sisters of the AAA patient.
The odds ratio for individual trials is shown as dots, error bars indicate 95% CIs.

and many enzymes involving the post-translational modification of structural proteins, the construction of the matrix and proteases involved in turnover of matrix components. Mutations in any of these candidate genes may explain the hereditary background of AAA. Therefore mutation analysis of all candidate genes is not an efficient approach. Moreover, we know from the classic hereditary connective tissue diseases, i.e. Marfan and Ehlers–Danlos type IV syndrome, that every family has its own gene mutation with typical clinical signs in affected individuals.

We reviewed the genetics on familial AAA, subdivided in genes involved in the structural components of the connective tissue of the aortic wall, genes of the matrix-degrading enzymes, their inhibitors, proteoglycans, and genes involved in the process of atherosclerosis.

Type III collagen

Collagen is the most common protein in humans, providing the extracellular framework for all multicellular organisms. The collagens are composed of a triple helix of three identical polypeptide α-chains, having a Gly-x-y repeating sequence, Gly means glycine, x proline, and y another amino acid.[31] Type III collagen is abundant in the vessel wall and responsible for the tensile strength of arteries.[32]

Abdominal aortic aneurysms, dissections and ruptures are common disorders in different syndromes that are caused by collagen defects. Phenotypic overlap of heritable disorders of connective tissue might be a possible explanation of familial aneurysms.[33] Ehlers–Danlos type IV syndrome (EDS IV), i.e. the vascular type, was first described by Barabas in 1967.[34] This type is characterised by a sudden death from rupture of the aorta and spontaneous dissections.

EDS IV is caused by genetically determined type III collagen (COL3A1) defect or a deficiency.

The hypothesis of familial AAA due to a type III collagen deficiency was first suggested by Loosemore *et al*. in 1987.[35] To study directly the role of the type III collagen gene Liu *et al*. produced type III collagen knock-out mice. Heterozygous mutant mice had about 50% reduction in type III collagen after protein analysis whereas no type III collagen was detected in homozygous mutant mice. The major cause of death in these mutant mice was rupture of a major blood vessel, similar to patients with EDS IV.[36] In patients with a family history for AAA lower amounts of type III collagen in the aortic media were measured in 6–18% of the patients.[23,37,38] However, gene mutations were detected even if the biosynthesis of type III procollagen was normal. Previously described type III procollagen gene mutations and the amino acid substitutions responsible for familial AAA are Gly619Arg, $G^{+1\ IVS20}$, Gly136Arg and Leu002Phe. These mutations influence the triple-helical domain of type III procollagen and make the protein unstable.[33,39–41] Another mutation, Thr501Pro, was found also in healthy controls, but without influence on the triple-helical domain of type III collagen.[41]

Additionally, an analysis of 50 patients indicated a causal relation with mutations in the type III procollagen in only 2% of aortic aneurysms.[33,39] Deak *et al*. measured an altered thermal stability of the type III collagen trimer also indicating that the triple helical conformation of type III collagen is imperfect.[42] Powell *et al*. could not confirm their previous claim that a mutation at amino acid 619 of glycine to arginine was associated with aneurysms. They suggested that the ageing aorta and changing mechanical stability of the artery might be influenced by variations in the type III collagen gene.[43–45] In 1999 our own group studied in a large family segregation of the type III collagen gene and the presence of AAA. One allele was found in all affected individuals and not in any of the unaffected relatives. This resulted in LOD (logarithm of the odds ratio) score of 1.5 with no recombination, so linkage with the type III collagen gene could not be proven or excluded.[23] In a subsequent study in family members of the AAA patients the protein analysis of cultured fibroblasts, used to measure the type III/I collagen ratio, was normal in all FDR. No evidence was found that a type III collagen deficiency appears to be an etiologic factor in the development of familial AAA.[30] Evidence for an increased metabolism of type III in AAA was provided by Bode *et al*. Immunohistochemical staining with antibodies for the amino-terminal propeptide of type III collagen (PIIINP), which represents newly synthesised type III collagen, was mainly present in the media layer of AAA. They suggested that the increased type III pN-collagen in AAA might result from impaired fibril formation causing aortic dilatation. In both AAA and atherosclerotic aorta's type I pN-collagen was present in the intima, which type is suggested possibly related to atherosclerotic changes.[46]

Sporadically, a type III collagen gene mutation is responsible for familial AAA. It is therefore more likely that collagen deficiency may result from defects during posttranslational modification or from an altered collagen metabolism.

Type I collagen, alpha 1 and 2

Type I collagen is a major constituent of the vessel wall. However, mutations in the type I collagen, alpha I (COL1A1) gene do not lead to vascular problems, except for easy bruising, but are the underlying cause of all types of osteogenesis imperfecta (OI).[47,48] Minion *et al*. reported an increase in type 1, alpha I, procollagen expression in AAA but not in atherosclerotic occlusive diseased or normal aortic tissue.[49] They gave no explanation for these matrix changes in the dilated aorta. There is no evidence available indicating that COL1A1 gene mutations are involved in aneurysm formation.

Mutations in the type I collagen, alpha 2 (COL1A2) gene are associated with OI and (rarely) forms of the EDS.[50–52] Vouyouka *et al*. demonstrated with a knock-out mouse model that type I collagen, alpha 2, is involved in the maintenance of biomechanical and functional properties of the aorta. Circumferential and longitudinal load-extension curves were used for determination of maximum breaking strength and incremental elastic modulus. The presence of homotrimeric type I collagen isotype (knock out mouse) significantly weakens the aorta. After histological analyses and hydroxyproline assays no significant differences were found in type I collagen content or organisation. The qualitative alterations appeared more important than the quantitative differences in collagen.[53] These data may help to elucidate the role of collagen in the development of aneurysmal aortic disease or dissection.

Elastin

Elastin is synthesised by smooth muscle cells and provides the elastic properties of the artery. It is organised in concentric lamellae and a progressive decrease in number of the lamellae is observed from the heart to the aorto-iliac bifurcation. Elastin is composed largely of glycine, proline, and other hydrophobic residues and contains multiple lysine-derived crosslinks, such as desmosines, which link the individual polypeptide

chains into a rubberlike network. The hydrophobic regions of the chains, situated between the crosslinks, are highly mobile.

Tropoelastin is the soluble precursor of elastin. The tropoelastin molecules are deposited into a microfibrillar structure that contain fibrillin-1 and 2 as the main components.[54] The amount of elastic tissue in the aortic wall appears to be gradually lost by ageing. However, the presence of low levels of tropoelastin mRNA in adult aorta's suggests that there is some continued elastin synthesis for growth and maintenance.[55,56] Many authors found a more pronounced decrease than can be attributed to aging.[56–59] A syndrome with mutations in the elastin gene is the Williams-Beuren syndrome (elfin facies), the phenotype is an autosomal dominant supravalvular aortic stenosis. This syndrome is not associated with AAA.[60,61] Boyd's research group (personal communication[62]) created a transgenic mouse with a deletion of exons 19–31 in the tropoelastin gene. The heterozygous founder suddenly died at 7 months of age. Autopsy findings were consistent with the hypothesis that death was due to rupture of an aneurysm of the aorta, suggesting that mutation in the gene of elastin may be associated with aneurysm disease in a transgenic model.[31] The long half-life of elastin and the low levels of tropoelastin mRNA may indicate that the loss of elastin is due to elastolysis. Summarising these studies, we can conclude that there is no clear evidence that mutations in the elastin gene are responsible for FAAA.

Fibrillin

The peripheral microfibrillar network that surrounds the core of the elastin largely consists of fibrillin and plays a critical role in the integrity of the aortic wall. Mutations in the fibrillin-1 gene are responsible for Marfan syndrome. The Marfan syndrome is a heritable disorder of connective tissue with skeletal, ocular and cardiovascular complications.[63]

Cardiovascular problems are mitral valve prolapse, aortic dilatation and aortic dissections. Nicod *et al*. and Milewicz *et al*. described families with aortic dissecting aneurysm or aortic dilatation at a young age of which none was related to the Marfan syndrome. They suggested a genetically determined disease of autosomal dominant inheritance with variable expression and reduced penetrance. The vascular complications were assumed probably caused by single gene mutation in absence of an associated syndrome.[64–66] In 1995 Francke *et al*. studied families with ascending aortic disease, aneurysms and dissection, none of them had Marfan syndrome. They found a Gly1127Ser mutation of the fibrillin-1 gene and suggested that this mutation might be responsible for reduced matrix deposition, with weakening of the elastic tissue. The clinical features of this amino acid substitution is less drastically than seen with substitutions of cysteine or other amino acids that cause a classic Marfan syndrome. This predisposes to ascending aortic dilatation later in life.[67] Powell *et al*. focused on blood pressure and fibrillin-1 genotype. Variation in the fibrillin-1 gene modulates the elastic properties of the ageing aorta which influences arterial pulse pressure. They postulated that the combination of the environmental risk factor, (untreated) hypertension and a genetic risk factor (variation in the fibrillin-1 gene) influences the development of aneurysmal disease.[45,68] By means of gene-targeting mutation of fibrillin-1 in a mouse model, Pereira *et al*. showed that an underexpression of fibrillin-1 could lead to an inflammatory fibroproliferative response and the formation of aortic aneurysms.[69] In summary it can be concluded that gene mutations in the fibrillin-1 gene are associated with familial thoracic aortic aneurysms (TAAA) and dissections, with no signs of a Marfan syndrome. The association with familial abdominal aortic aneurysms needs more profound investigation.

Matrix proteinases and tissue inhibitor metalloproteinases

The matrix metalloproteinases (MMPs) are a family of related matrix-degrading enzymes that are important in tissue remodelling and repair. Abnormal expression is associated with various diseases such as tumor invasiveness, inflammatory processes and atherosclerosis. Enzymatic degradation of structural matrix proteins is said to contribute to the pathogenesis of AAA.[70–76] Activated MMPs weaken the aortic media, as they cause destruction of elastic fibres and smooth

Table 2. MMPs expressed and produced in the inflammatory process in the atherosclerotic aneurysmal wall and the substrates.

Enzyme	Substrate
MMP1 (interstitial collagenase)	Collagen type I and III
MMP3 (stromelysin)	Proteoglycans, laminin, fibronectin, collagen type IV, V, IX, and X, and enhances activity of MMP1
MMP2 (72-kD gelatinase/ gelatinase A)	Denatured collagen, collagen type
MMP9 (92-kD gelatinase/ gelatinase B)	IV, V, VII, and X, and elastin
MMP7 (matrilysin)	Gelatin, laminin, fibronectin, type IV collagen, versican, and elastin
MMP12 (macrophage metalloelastase)	Elastin

MMP means matrix metalloproteinase.

muscle cells.[37,58] Most of the MMPs are expressed in macrophages, the major inflammatory cells responsible for matrix degradation. Busutill *et al*. first mentioned elastase and collagenase activity in the human aorta.[77,78] Table 2 recite the most expressed and produced MMPs in the inflammatory process in the atherosclerotic aneurysmal wall.[71,79–84]

The MMPs are acting as a cascade, starting with MMP1, to mediate the degradation of aortic wall elastic fibres.[85] Increased expression of MMP9 is measured in AAA at both the messenger RNA and protein levels.[86,87] MMP9 levels in plasma are associated with size and expansion of the AAA.[72] Studies using cDNA microarrays reported an upregulation of MMP9 gene expression in AAA versus healthy aorta. MMP9 was also upregulated in atherosclerotic aorta.[88,89] MMP9 is localised to the adventitia and adventitial side of the tunica media in AAA tissue and in the intima and intimal side of the media in atherosclerotic occlusive aorta tissue.[90] There is evidence, although not conclusive, that the 5A allele of MMP3 is a risk factor for AAA among Finnish patients.[91] Studies reported increased production of MMP13, MMP2, MMP12 and membrane type-1 (MT-1) MMP in human aneurysmal tissue compared to atherosclerotic-and normal aortic tissue.[70,92–94] MT-1 MMP activates pro-MMP2, which is proteolytically processed to the MMP2 active form. Increased levels of MMP13, 2 and 12 play a role in the collagen and elastin degradation and therefore they could have a role in aneurysmal disease.

The serine proteases include plasmin, thrombin, urokinase-(u-PA) and tissue-type-plasminogen activator (t-PA). Plasmin is activated by u-PA and t-PA and activates MMP1 and MMP9. Leakage of plasmin from a large mural thrombus to the AAA may lead to accelerated matrix destruction and rapid aneurysm enlargement.[95]

Rossaak *et al*. found in patients with familial AAA, a significantly different allele frequency for plasminogen activator inhibitor-1 (PAI-1; 4G-allele) compared with non-familial AAA patients and controls. The 4G homozygous variant is associated with increased PAI-1 expression and consequently reduced plasmin activity and therefore may be selected against in-familial AAA.[96] These results were in contrast with that reported by Yoon *et al*., who found no significant difference, between AAA patients and controls in PAI-1 genotype and allele frequencies.[91]

Because apolipoprotein E (apoE) is a ligand for receptors that clears remnants of chylomicrons and very low density lipoproteins, lack of apoE would be expected to cause accumulation in plasma of cholesterol-rich remnants which prolonged circulation could be atherogenic.[97–99] Gerdes *et al*. suggest that the common polymorphism of the apoE genotype influences the AAA expansion rate.[100] ApoE and u-PA deficient mice were protected for aneurysm formation, probably because there was less plasmin to activate the pro-MMPs.[101]

Cysteine proteases, i.e. cathepsin S and K, regulate the intracellular protein degradation and turnover.[85] They are localised in smooth muscle cells and macrophages within atherosclerotic plaques.[102] Lysosomal proteases, i.e. cathepsin D, H and L, are involved in the degradation of structural proteins and were found to have higher activities in aneurysmal wall and mural thrombus than in the normal aortic wall.[88,103] These cathepsins can mediate extensive matrix breakdown, thereby negatively influencing the elasticity and mechanical resistance of the aortic wall.

The most abundant extracellular inhibitor of the cysteine proteases is cystatin C. Increased aneurysm size and expansion rate are associated with cystatin C deficiency.[104]

The tissue inhibitors of metalloproteinases (TIMPs) are major inhibitors of several enzymes that are destructive to connective tissue. Brophy *et al*. reported a decrease of TIMP in AAA tissue. This may contribute to the increased proteolysis observed in AAA.[105] Quantifying relative mRNA levels resulted in a higher ratio of MMP mRNA amount to TIMP mRNA in AAA than seen in normal aortas.[106] No mutations were found in the TIMP 1 or 2 genes in aneurysm patients.[107,108]

Several cytokines, i.e. interleukin-1β, tumor necrosis factor-α, and interleukin-6, are suggested influencing the integrity of the aortic wall resulting in degenerative as well as inflammatory aneurysms.[84,94,109–111] Interleukin-1β causes an increased matrix turnover in aneurysms through its effect on smooth muscle cell collagenase and collagen gene expression.[76] Due to the inflammatory component, elastin fragments trigger the migration of macrophages leading to interleukin-1β production, which can change the gene products of the aortic smooth muscle cells leading to collagenase production. Collagenase activity is detectable in the ruptured aneurysm wall.[38,78]

The concentration of interleukin-6 is influenced by the genotype. In patients with small AAA, the 174G/C polymorphism of the interleukin-6 gene predicts future cardiovascular mortality.[111] It remains difficult to establish whether the higher circulating concentrations of cytokines are caused by or a consequence of AAA or may be due to other causes, i.e. malignancy, infection or atherosclerosis.

The immune response within the aortic wall is regulated by the human leukocyte antigen (HLA) class II genes. HLA alleles have been suggested to act as genetic risk factors for AAA.[112,113] Hirose *et al*. suggests that HLA-DQ3 antigen appeared to have a protective effect[114] in relation to AAA, whereas HLA DR15 actually promotes AAA disease.[115]

Recently Unno *et al*. detected more gene mutations in plasma platelet activating factor (PAF) acetylhydrolase in Japanese AAA patients than in controls. The authors hypothesize that deficiency of PAF acetylhydrolase may fail to inactivate PAF, thereby accelerating inflammation and MMP production, which may contribute to the development of AAA.[116]

There is ample evidence that the degradation of extracellular matrix proteins leads to aneurysm formation of the abdominal aorta. The question remains if there is a specific MMP responsible for aneurysm formation. More likely this process is caused by a variety of proteins in the cascade of matrix turnover.

Other candidate genes

Genes of haptoglobin alpha chain (HP) and cholesterol-ester transfer protein (CETP) are located on the long arm of chromosome 16. Variations at the HP locus might have a direct effect on the degradation of elastin in the atherosclerotic aorta, whereas variations at the CETP locus could affect the lipoprotein metabolism and might therefore alter the susceptibility to atherosclerosis. Powell *et al*. first mentioned that HPs containing an alpha 1-chain accelerate the degradation by elastases of aortic elastin in vitro two- to four-fold. An increase of the frequency of a rare polymorphism at the CETP locus in aneurysm patients suggests that variations in these proteins influences dilatation of the abdominal aorta.[117] Later they were unable to confirm that HP might be informative as a marker for screening in familial AAA.[43] Also Rambottom *et al*. were not able to detect CETP and HP microsatellite polymorphism.[118]

Kuivaniemi *et al*. findings exclude mutations in the coding sequence of fibulin 2, an extracellular matrix protein, as a major cause of AAA, but they suggest that fibulin-2 exhibits a high degree of sequence variability.[119]

Alpha1-Antitrypsin, an elastase inhibitor, could not be detected as a gene-disease marker in plasma.[118] No relation was found with alpha1-antitrypsin deficiency and AAA using monoclonal-antibody ELISA tests.[120]

Biglycan and decorin are proteoglycans, found in many connective tissues, are synthesised by smooth muscle cells. They play a role in stabilising the structure of collagen fibres. Changes in the production and/or the function of these proteoglycans could be responsible for matrix alterations and weakening of the vascular wall. Tamarina *et al*. found a 15-fold decrease in biglycan mRNA expression in AAA compared with normal aortas. The decorin mRNA expression was unchanged.[87] Tung *et al*. found an increased decorin expression whereas Armstrong *et al*. found no changed expression in their gene array evaluation of AAA.[88,89] In contrast Melrose *et al*. found elevated levels of biglycan and other heparan sulfate proteoglycans in smooth muscle culture of AAA tissue.[121] It is disputable whether biglycan plays an important role in the integrity of the aortic wall. Xu *et al*. generated biglycan deficient mice that seemed normal at birth but displayed a phenotype characterised by reduced growth rate and decreased bone mass, but no vascular abnormalities were found.[122]

Hyperhomocysteinemia due to alterations in the homocysteine metabolism is a risk factor for (premature) atherosclerotic disease.[123] Brunelli *et al*. measured elevated homocysteine levels in patients with and without evidence of atherosclerotic localisations compared with controls. A mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) coding sequence has been associated with reduced specific MTHFR activity and elevated homocysteine levels.[124] The genotype distribution of the C677T MTHFR mutation was not significantly different in the AAA patients compared with controls.[125]

Linkage analysis

The candidate gene approach might give us more insight in the pathology of AAA but does not lead to the specific genetic factor(s) responsible for (familial) AAA. A disadvantage of candidate gene approach is that the investigated proteins are functioning in a very complex process of construction and degradation in the ageing abdominal aortic wall. Pathological synthesis of one of these components may result from defects during post-translational modification or from an altered protein metabolism. On the other hand, linkage analysis gives an indication of chromosomal localisation, but no information on the function. Linkage of a disease phenotype, with a DNA marker of known chromosomal location, means that the two are located in close proximity on the DNA. Cross-overs happen most of the time during meiosis. Meiotic recombination frequencies of less than 50% indicate that the disease phenotype and the marker are linked. Linkage can be established whenever the phenotype and genotype of related individuals is analysed, therefore linkage can be established by analysing large families or by analysing pairs of siblings. AAA is a late onset

disease and in most cases only one generation is affected, because the parents have died and the children are too young to develop an AAA. A further problem is that siblings of AAA patients can develop AAA later in life. To eliminate the problem of late-onset disease an affected sib-pair analysis can be performed.[126] Linked loci are further examined to detect the genes involved in the pathology of familial AAA.

Guo *et al*. performed linkage analysis of patients with familial thoracic aortic aneurysms (TAA) and dissections. A major locus for familial TAA and dissections mapped to chromosome 5q13-14, with the majority of the families (9 of 15) identified, demonstrating evidence of linkage to this locus. Linkage analysis of the other six families did not demonstrate evidence of a linkage to any loci previously associated with aneurysm formation.[127]According to the authors, genes mapped to the chromosome 5q13-14 region are thought to be poor candidates for the defective gene involved in TAAs/dissections because of there function. In our opinion, in this region the betaine-homocysteine methyltransferase gene may be a possible candidate. Betaine-homocysteine methyltransferase catalyses the conversion of betaine and homocysteine to dimethylglycine and methionine. Hyperhomocysteinemia is a risk factor for arterial occlusive disease, but probably also for aneurysmal disease.[123,125,128,129]

Scientists from Wayne State University in Detroit, in collaboration with scientists from other universities, already started an affected sib-pair DNA linkage analysis to identify AAA susceptible gene(s).[130]

Conclusion

The siblings of AAA patients, especially among the brothers, are significantly more affected, supporting that AAA can be an inheritable disease.

A lot of questions in the genetics of familial AAA remain unanswered. The inheritance is most likely to be autosomal dominant with incomplete penetrance. Therefore deficiencies or gene mutations of proteins of the structural components of the connective tissue are likely responsible for susceptibility to AAA. Defects during post-translational modification or from an altered protein metabolism are possible alternative explanations, but enzyme deficiencies are more likely to show recessive inheritance. AAA is a possible genetically heterogeneous condition. A small number of families have a mutation in COL3A1, indicating that AAA in some cases may be considered a subclinical manifestation of EDS IV. Disturbed regulation of proteases, i.e. MMP2, 3 and 9, influencing the balance in tissue remodelling and repair, must be considered as an important factor in the pathogenesis of AAA. More studies need to be done to investigate if changes in gene expression, activation of the proenzyme forms of the MMP's or competition with the TIMP's are responsible for the imbalance in tissue architecture. In the future, linkage analysis, may resolve the genetic background of AAA. Genetic studies may define subgroups of patients at risk for familial AAA who would benefit most from ultrasound screenings programmes.

References

1 Collaborative Aneurysm Screening Study Group (CASS Group): Ashton H, Couto E, Duffy S, Jamrozik K, Lindholt J. A comparative study of the prevalence of abdominal aortic aneurysms in the United Kingdom, Denmark, and Australia. *J Med Screen* 2001; **8**: 46–50.

2 Boll AP, Verbeek AL, van de Lisdonk EH *et al*. High prevalence of abdominal aortic aneurysm in a primary care screening programme. *Br J Surg* 1998; **85**: 1090–1094.

3 Wilmink AB, Hubbard CS, Day NE *et al*. The incidence of small abdominal aortic aneurysms and the change in normal infrarenal aortic diameter: implications for screening. *Eur J Vasc Endovasc Surg* 2001; **21**: 165–170.

4 Kahn CE, Jr, Quiroz FA. Positive predictive value of clinical suspicion for abdominal aortic aneurysm. Implications for use of ultrasonography [see comments]. *J Gen Intern Med* 1996; **11**: 756–758.

5 Wilmink AB, Pleumeekers HJ, Hoes AW *et al*. The infrarenal aortic diameter in relation to age: only part of the population in older age groups shows an increase. *Eur J Vasc Endovasc Surg* 1998; **16**: 431–437.

6 Boll AP, van der Vliet JA. Abdominal aortic aneurysm: a growing problem. *Cardiologia* 1998; **43**: 571–575.

7 Vardulaki KA, Walker NM, Day NE *et al*. Quantifying the risks of hypertension, age, sex and smoking in patients with abdominal aortic aneurysm. *Br J Surg* 2000; **87**: 195–200.

8 The UK Small Aneurysm Trial Participants. Smoking, lung function and the prognosis of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 2000; **19**: 636–642.

9 Lederle FA, Johnson GR, Wilson SE *et al*. The aneurysm detection and management study screening program: validation cohort and final results. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. *Arch Intern Med* 2000; **160**: 1425–1430.

10 Lederle FA, Johnson GR, Wilson SE *et al*. Prevalence and associations of abdominal aortic aneurysm detected through screening. Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. *Ann Intern Med* 1997; **126**: 441–449.

11 Wilmink TB, Quick CR, Day NE. The association between cigarette smoking and abdominal aortic aneurysms. *J Vasc Surg* 1999; **30**: 1099–1105.

12 Wilmink AB, Quick CR. Epidemiology and potential for prevention of abdominal aortic aneurysm. *Br J Surg* 1998; **85**: 155–162.

13 Powell JT, Worrell P, MacSweeney ST *et al*. Smoking as a risk factor for abdominal aortic aneurysm. *Ann NY Acad Sci* 1996; **800**: 246–248.

14 Clifton MA. Familial abdominal aortic aneurysms. *Br J Surg* 1977; **64**: 765–766.

15 Norrgard O, Rais O, Angquist KA. Familial occurrence of abdominal aortic aneurysms. *Surgery* 1984; **95**: 650–656.

16 Johansen K, Koepsell T. Familial tendency for abdominal aortic aneurysms. *JAMA* 1986; **256**: 1934–1936.
17 Powell JT, Greenhalgh RM. Multifactorial inheritance of abdominal aortic aneurysm. *Eur J Vasc Surg* 1987; **1**: 29–31.
18 Darling RC, Brewster DC, LaMuraglia GM *et al*. Are familial abdominal aortic aneurysms different? *J Vasc Surg* 1989; **10**: 39–43.
19 Collin J, Walton J. Is abdominal aortic aneurysm familial? [see comments]. *BMJ* 1989; **299**: 493.
20 Webster MW, Ferrell RE, St Jean PL *et al*. Ultrasound screening of first-degree relatives of patients with an abdominal aortic aneurysm. *J Vasc Surg* 1991; **13**: 9–13.
21 Baird PA, Sadovnick AD, Yee IM *et al*. Sibling risks of abdominal aortic aneurysm. *Lancet* 1995; **346**: 601–604.
22 Jaakkola P, Kuivaniemi H, Partanen K *et al*. Familial abdominal aortic aneurysms: screening of 71 families. *Eur J Surg* 1996; **162**: 611–617.
23 van Keulen CJ, van de AE, Pals G *et al*. The role of type III collagen in the development of familial abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 1999; **18**: 65–70.
24 Rossaak JI, Hill TM, Jones GT *et al*. Familial abdominal aortic aneurysms in the Otago region of New Zealand. *Cardiovasc Surg* 2001; **9**: 241–248.
25 Salo JA, Soisalon-soininen S, Bondestam S *et al*. Familial occurrence of abdominal aortic aneurysm. *Ann Intern Med* 1999; **130**: 637–642.
26 Tilson MD, Seashore MR. Fifty families with abdominal aortic aneurysms in two or more first-order relatives. *Am J Surg* 1984; **147**: 551–553.
27 Falconer DS. The inheritance of liability to diseases with variable age of onset, with particular reference to diabetes mellitus. *Ann Hum Genet* 1967; **31**: 1–20.
28 Majumder PP, St Jean PL, Ferrell RE *et al*. On the inheritance of abdominal aortic aneurysm. *Am J Hum Genet* 1991; **48**: 164–170.
29 Verloes A, Sakalihasan N, Koulischer L *et al*. Aneurysms of the abdominal aorta: familial and genetic aspects in three hundred thirteen pedigrees. *J Vasc Surg* 1995; **21**: 646–655.
30 van Keulen CJ, van den AE, van den Berg FG *et al*. The role of type III collagen in family members of patients with abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2000; **20**: 379–385.
31 Ghorpade A, Baxter BT. Biochemistry and molecular regulation of matrix macromolecules in abdominal aortic aneurysms. *Ann NY Acad Sci* 1996; **800**: 138–150.
32 Vandenberg P. Molecular basis of heritable connective tissue disease. *Biochem Med Metab Biol* 1993; **49**: 1–12.
33 Kontusaari S, Tromp G, Kuivaniemi H *et al*. Inheritance of an RNA splicing mutation (G^{+1} IVS20) in the type III procollagen gene (COL3A1) in a family having aortic aneurysms and easy bruisability: phenotypic overlap between familial arterial aneurysms and Ehlers-Danlos syndrome type IV. *Am J Hum Genet* 1990; **47**: 112–120.
34 Barabas AP. Heterogeneity of the Ehlers–Danlos syndrome: description of three clinical types and a hypothesis to explain the basic defect(s). *Br Med J* 1967; **2**: 612–613.
35 Loosemore TM, Child AH, Dormandy JA. Familial abdominal aortic aneurysms. *J R Soc Med* 1988; **81**: 472–473.
36 Liu X, Wu H, Byrne M *et al*. Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. *Proc Natl Acad Sci USA* 1997; **94**: 1852–1856.
37 Powell J, Greenhalgh RM. Cellular, enzymatic, and genetic factors in the pathogenesis of abdominal aortic aneurysms. *J Vasc Surg* 1989; **9**: 297–304.
38 Menashi S, Campa JS, Greenhalgh RM *et al*. Collagen in abdominal aortic aneurysm: typing, content, and degradation. *J Vasc Surg* 1987; **6**: 578–582.
39 Kontusaari S, Tromp G, Kuivaniemi H *et al*. A mutation in the gene for type III procollagen (COL3A1) in a family with aortic aneurysms. *J Clin Invest* 1990; **86**: 1465–1473.
40 Tromp G, Wu Y, Prockop DJ *et al*. Sequencing of cDNA from 50 unrelated patients reveals that mutations in the triple-helical domain of type III procollagen are an infrequent cause of aortic aneurysms. *J Clin Invest* 1993; **91**: 2539–2545.
41 Anderson DW, Edwards TK, Ricketts MH *et al*. Multiple defects in type III collagen synthesis are associated with the pathogenesis of abdominal aortic aneurysms. *Ann NY Acad Sci* 1996; **800**: 216–228.
42 Deak SB, Ricotta JJ, Mariani TJ *et al*. Abnormalities in the biosynthesis of type III procollagen in cultured skin fibroblasts from two patients with multiple aneurysms. *Matrix* 1992; **12**: 92–100.
43 Adamson J, Powell JT, Greenhalgh RM. Selection for screening for familial aortic aneurysms. *Br J Surg* 1992; **79**: 897–898.
44 Powell JT, Adamson J, MacSweeney ST *et al*. Influence of type III collagen genotype on aortic diameter and disease. *Br J Surg* 1993; **80**: 1246–1248.
45 MacSweeney ST, Skidmore C, Turner RJ *et al*. Unravelling the familial tendency to aneurysmal disease: popliteal aneurysm, hypertension and fibrillin genotype. *Eur J Vasc Endovasc Surg* 1996; **12**: 162–166.
46 Bode MK, Soini Y, Melkko J *et al*. Increased amount of type III pN-collagen in human abdominal aortic aneurysms: evidence for impaired type III collagen fibrillogenesis. *J Vasc Surg* 2000; **32**: 1201–1207.
47 Pope FM, Nicholls AC, McPheat J *et al*. Collagen genes and proteins in osteogenesis imperfecta. *J Med Genet* 1985; **22**: 466–478.
48 Kuivaniemi H, Tromp G, Prockop DJ. Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. *Hum Mutat* 1997; **9**: 300–315.
49 Minion DJ, Wang Y, Lynch TG *et al*. Soluble factors modulate changes in collagen gene expression in abdominal aortic aneurysms. *Surgery* 1993; **114**: 252–256.
50 Superti-Furga A, Pistone F, Romano C *et al*. Clinical variability of osteogenesis imperfecta linked to COL1A2 and associated with a structural defect in the type I collagen molecule. *J Med Genet* 1989; **26**: 358–362.
51 Minor RR, Sippola-Thiele M, McKeon J *et al*. Defects in the processing of procollagen to collagen are demonstrable in cultured fibroblasts from patients with the Ehlers–Danlos and osteogenesis imperfecta syndromes. *J Biol Chem* 1986; **261**: 10006–10014.
52 Sasaki T, Arai K, Ono M *et al*. Ehlers–Danlos syndrome. A variant characterized by the deficiency of pro alpha 2 chain of type I procollagen. *Arch Dermatol* 1987; **123**: 76–79.
53 Vouyouka AG, Pfeiffer BJ, Liem TK *et al*. The role of type I collagen in aortic wall strength with a homotrimeric. *J Vasc Surg* 2001; **33**: 1263–1270.
54 Verloes A, Sakalihasan N, Limet R *et al*. Genetic aspects of abdominal aortic aneurysm. *Ann NY Acad Sci* 1996; **800**: 44–55.
55 Rucker RB, Tinker D. Structure and metabolism of arterial elastin. *Int Rev Exp Pathol* 1977; **17**: 1–47.
56 Mesh CL, Baxter BT, Pearce WH *et al*. Collagen and elastin gene expression in aortic aneurysms. *Surgery* 1992; **112**: 256–261.
57 Sakalihasan N, Heyeres A, Nusgens BV *et al*. Modifications of the extracellular matrix of aneurysmal abdominal aortas as a function of their size. *Eur J Vasc Surg* 1993; **7**: 633–637.
58 He CM, Roach MR. The composition and mechanical properties of abdominal aortic aneurysms. *J Vasc Surg* 1994; **20**: 6–13.
59 Rizzo RJ, McCarthy WJ, Dixit SN *et al*. Collagen types and matrix protein content in human abdominal aortic aneurysms. *J Vasc Surg* 1989; **10**: 365–373.
60 Ewart AK, Jin W, Atkinson D *et al*. Supravalvular aortic stenosis associated with a deletion disrupting the elastin gene. *J Clin Invest* 1994; **93**: 1071–1077.
61 Olson TM, Michels VV, Urban Z *et al*. A 30 kb deletion within the elastin gene results in familial supravalvular aortic stenosis. *Hum Mol Genet* 1995; **4**: 1677–1679.
62 Tilson MD. Abdominal aortic aneurysm surgery: something old, something new. *Cardiovasc Surg* 1994; **2**: 159–163.

63 Pyeritz RE. The Marfan syndrome. *Annu Rev Med* 2000; **51**: 481–510.
64 Milewicz DM, Chen H, Park ES *et al*. Reduced penetrance and variable expressivity of familial thoracic aortic aneurysms/dissections. *Am J Cardiol* 1998; **82**: 474–479.
65 Milewicz DM, Michael K, Fisher N *et al*. Fibrillin-1 (FBN1) mutations in patients with thoracic aortic aneurysms. *Circulation* 1996; **94**: 2708–2711.
66 Nicod P, Bloor C, Godfrey M *et al*. Familial aortic dissecting aneurysm. *J Am Coll Cardiol* 1989; **13**: 811–819.
67 Francke U, Berg MA, Tynan K *et al*. A Gly1127Ser mutation in an EGF-like domain of the fibrillin-1 gene is a risk factor for ascending aortic aneurysm and dissection. *Am J Hum Genet* 1995; **56**: 1287–1296.
68 Powell JT, MacSweeney ST, Greenhalgh RM *et al*. Interaction between fibrillin genotype and blood pressure and the development of aneurysmal disease. *Ann N Y Acad Sci* 1996; **800**: 198–207.
69 Pereira L, Lee SY, Gayraud B *et al*. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc Natl Acad Sci USA* 1999; **96**: 3819–3823.
70 Crowther M, Goodall S, Jones JL *et al*. Increased matrix metalloproteinase 2 expression in vascular smooth muscle cells cultured from abdominal aortic aneurysms. *J Vasc Surg* 2000; **32**: 575–583.
71 Thompson RW, Parks WC. Role of matrix metalloproteinases in abdominal aortic aneurysms. *Ann N Y Acad Sci* 1996; **800**: 157–174.
72 Lindholt JS, Vammen S, Fasting H *et al*. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms. A preliminary study. *Eur J Vasc Endovasc Surg* 2000; **20**: 281–285.
73 Sakalihasan N, Delvenne P, Nusgens BV *et al*. Activated forms of MMP2 and MMP9 in abdominal aortic aneurysms. *J Vasc Surg* 1996; **24**: 127–133.
74 Crowther M, Goodall S, Jones JL *et al*. Localization of matrix metalloproteinase 2 within the aneurysmal and normal aortic wall. *Br J Surg* 2000; **87**: 1391–1400.
75 Nakamura M, Tachieda R, Niinuma H *et al*. Circulating biochemical marker levels of collagen metabolism are abnormal in patients with abdominal aortic aneurysm. *Angiology* 2000; **51**: 385–392.
76 Keen RR, Nolan KD, Cipollone M *et al*. Interleukin-1 beta induces differential gene expression in aortic smooth muscle cells. *J Vasc Surg* 1994; **20**: 774–784.
77 Busuttil RW, Rinderbriecht H, Flesher A *et al*. Elastase activity: the role of elastase in aortic aneurysm formation. *J Surg Res* 1982; **32**: 214–217.
78 Busuttil RW, Abou-Zamzam AM, Machleder HI. Collagenase activity of the human aorta. A comparison of patients with and without abdominal aortic aneurysms. *Arch Surg* 1980; **115**: 1373–1378.
79 Mao D, VanVickle SJ, Curci JA *et al*. Expression of matrix metalloproteinases and TIMPs in human abdominal aortic aneurysms. *Ann Vasc Surg* 1999; **13**: 236–237.
80 Vine N, Powell JT. Metalloproteinases in degenerative aortic disease. *Clin Sci (Colch)* 1991; **81**: 233–239.
81 Newman KM, Ogata Y, Malon AM *et al*. Identification of matrix metalloproteinases 3 (stromelysin-1) and 9 (gelatinase B) in abdominal aortic aneurysm. *Arterioscler Thromb* 1994; **14**: 1315–1320.
82 Newman KM, Malon AM, Shin RD *et al*. Matrix metalloproteinases in abdominal aortic aneurysm: characterization, purification, and their possible sources. *Connect Tissue Res* 1994; **30**: 265–276.
83 Elmore JR, Keister BF, Franklin DP *et al*. Expression of matrix metalloproteinases and TIMPs in human abdominal aortic aneurysms. *Ann Vasc Surg* 1998; **12**: 221–228.
84 Shah PK. Inflammation, metalloproteinases, and increased proteolysis: an emerging pathophysiological paradigm in aortic aneurysm. *Circulation* 1997; **96**: 2115–2117.
85 Mauch C. Regulation of connective tissue turnover by cell-matrix interactions. *Arch Dermatol Res* 1998; **290 (Suppl)**: S30–S36.
86 Pyo R, Lee JK, Shipley JM *et al*. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J Clin Invest* 2000; **105**: 1641–1649.
87 Tamarina NA, Grassi MA, Johnson DA *et al*. Proteoglycan gene expression is decreased in abdominal aortic aneurysms. *J Surg Res* 1998; **74**: 76–80.
88 Tung WS, Lee JK, Thompson RW. Simultaneous analysis of 1176 gene products in normal human aorta and abdominal aortic aneurysms using a membrane-based complementary DNA expression array. *J Vasc Surg* 2001; **34**: 143–150.
89 Armstrong PJ, Johanning JM, Calton WC, Jr *et al*. Differential gene expression in human abdominal aorta: aneurysmal versus occlusive disease. *J Vasc Surg* 2002; **35**: 346–355.
90 Palombo D, Maione M, Cifiello BI *et al*. Matrix metalloproteinases. Their role in degenerative chronic diseases of abdominal aorta. *J Cardiovasc Surg (Torino)* 1999; **40**: 257–260.
91 Yoon S, Tromp G, Vongpunsawad S *et al*. Genetic analysis of MMP3, MMP9, and PAI-1 in Finnish patients with abdominal aortic or intracranial aneurysms. *Biochem Biophys Res Commun* 1999; **265**: 563–568.
92 Mao D, Lee JK, VanVickle SJ *et al*. Expression of collagenase-3 (MMP-13) in human abdominal aortic aneurysms and vascular smooth muscle cells in culture. *Biochem Biophys Res Commun* 1999; **261**: 904–910.
93 Nollendorfs A, Greiner TC, Nagase H *et al*. The expression and localization of membrane type-1 matrix metalloproteinase in human abdominal aortic aneurysms. *J Vasc Surg* 2001; **34**: 316–322.
94 Curci JA, Liao S, Huffman MD *et al*. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. *J Clin Invest* 1998; **102**: 1900–1910.
95 Jean-claude J, Newman KM, Li H *et al*. Possible key role for plasmin in the pathogenesis of abdominal aortic aneurysms. *Surgery* 1994; **116**: 472–478.
96 Rossaak JI, Van Rij AM, Jones GT *et al*. Association of the 4G/5G polymorphism in the promoter region of plasminogen activator inhibitor-1 with abdominal aortic aneurysms. *J Vasc Surg* 2000; **31**: 1026–1032.
97 Davignon J, Cohn JS, Mabile L *et al*. Apolipoprotein E and atherosclerosis: insight from animal and human studies. *Clin Chim Acta* 1999; **286**: 115–143.
98 Linton MF, Atkinson JB, Fazio S. Prevention of atherosclerosis in apolipoprotein E-deficient mice by bone marrow transplantation. *Science* 1995; **267**: 1034–1037.
99 Weisgraber KH. Apolipoprotein E: structure-function relationships. *Adv Protein Chem* 1994; **45**: 249–302.
100 Gerdes LU, Lindholt JS, Vammen S *et al*. Apolipoprotein E genotype is associated with differential expansion rates of small abdominal aortic aneurysms. *Br J Surg* 2000; **87**: 760–765.
101 Carmeliet P, Moons L, Lijnen R *et al*. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nat Genet* 1997; **17**: 439–444.
102 Sukhova GK, Shi GP, Simon DI *et al*. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest* 1998; **102**: 576–583.
103 Gacko M, Glowinski S. Cathepsin D and Cathepsin L activities in aortic aneurysm wall and parietal thrombus. *Clin Chem Lab Med* 1998; **36**: 449–452.
104 Lindholt JS, Erlandsen EJ, Henneberg EW. Cystatin C deficiency is associated with the progression of small abdominal aortic aneurysms. *Br J Surg* 2001; **88**: 1472–1475.
105 Brophy CM, Marks WH, Reilly JM *et al*. Decreased tissue inhibitor of metalloproteinases (TIMP) in abdominal aortic aneurysm tissue: a preliminary report. *J Surg Res* 1991; **50**: 653–657.

106 Tamarina NA, McMillan WD, Shively VP *et al*. Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta. *Surgery* 1997; **122**: 264–271.
107 Wang X, Tromp G, Cole CW *et al*. Analysis of coding sequences for tissue inhibitor of metalloproteinases 1 (TIMP1) and 2 (TIMP2) in patients with aneurysms. *Matrix Biol* 1999; **18**: 121–124.
108 Tilson MD, Reilly JM, Brophy CM *et al*. Expression and sequence of the gene for tissue inhibitor of metalloproteinases in patients with abdominal aortic aneurysms. *J Vasc Surg* 1993; **18**: 266–270.
109 Rijbroek A, Moll FL, van Dijk HA *et al*. Inflammation of the abdominal aortic aneurysm wall. *Eur J Vasc Surg* 1994; **8**: 41–46.
110 Juvonen J, Surcel HM, Satta J *et al*. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2843–2847.
111 Jones KG, Brull DJ, Brown LC *et al*. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. *Circulation* 2001; **103**: 2260–2265.
112 Tilson MD, Ozsvath KJ, Hirose H *et al*. A genetic basis for autoimmune manifestations in the abdominal aortic aneurysm resides in the MHC class II locus DR-beta-1. *Ann NY Acad Sci* 1996; **800**: 208–215.
113 Rasmussen TE, Hallett JW, Jr, Schulte S *et al*. Genetic similarity in inflammatory and degenerative abdominal aortic aneurysms: a study of human leukocyte antigen class II disease risk genes. *J Vasc Surg* 2001; **34**: 84–89.
114 Hirose H, Tilson MD. Negative genetic risk factor for abdominal aortic aneurysm: HLA-DQ3, a Japanese study. *J Vasc Surg* 1999; **30**: 959–960.
115 Hirose H, Takagi M, Miyagawa N *et al*. Genetic risk factor for abdominal aortic aneurysm: HLA-DR2(15), a Japanese study. *J Vasc Surg* 1998; **27**: 500–503.
116 Unno N, Nakamura T, Mitsuoka H *et al*. Association of a g994 → t missense mutation in the plasma platelet-activating factor acetylhydrolase gene with risk of abdominal aortic aneurysm in Japanese. *Ann Surg* 2002; **235**: 297–302.
117 Powell JT, Bashir A, Dawson S *et al*. Genetic variation on chromosome 16 is associated with abdominal aortic aneurysm. *Clin Sci (Colch)* 1990; **78**: 13–16.
118 Ramsbottom D, Fitzgerald P, Grace PA *et al*. Biochemical and molecular genetic studies of abdominal aortic aneurysm in an Irish population. *Eur J Vasc Surg* 1994; **8**: 716–722.
119 Kuivaniemi H, Marshall A, Ganguly A *et al*. Fibulin-2 exhibits high degree of variability, but no structural changes concordant with abdominal aortic aneurysms. *Eur J Hum Genet* 1998; **6**: 642–646.
120 Elzouki AN, Ryden AA, Lanne T *et al*. Is there a relationship between abdominal aortic aneurysms and alpha1-antitrypsin deficiency (PiZ)? *Eur J Vasc Endovasc Surg* 1999; **17**: 149–154.
121 Melrose J, Whitelock J, Xu Q *et al*. Pathogenesis of abdominal aortic aneurysms: possible role of differential production of proteoglycans by smooth muscle cells. *J Vasc Surg* 1998; **28**: 676–686.
122 Xu T, Bianco P, Fisher LW *et al*. Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. *Nat Genet* 1998; **20**: 78–82.
123 Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998; **338**: 1042–1050.
124 Frosst P, Blom HJ, Milos R *et al*. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10**: 111–113.
125 Brunelli T, Prisco D, Fedi S *et al*. High prevalence of mild hyperhomocysteinemia in patients with abdominal aortic aneurysm. *J Vasc Surg* 2000; **32**: 531–536.
126 Tromp G, Kuivaniemi H. DNA sequencing as a method to identify mutations in patients with familial forms of aneurysms. *J Vasc Surg* 1992; **15**: 928–930.
127 Guo D, Hasham S, Kuang SQ *et al*. Familial thoracic aortic aneurysms and dissections: genetic heterogeneity with a major locus mapping to 5q13-14. *Circulation* 2001; **103**: 2461–2468.
128 Caldwell S. Hyperhomocysteinaemia. *Eur J Vasc Endovasc Surg* 1998; **16**: 171–172.
129 Mohan IV, Adam DJ, Kurian KM *et al*. Isolated external iliac aneurysm associated with hyperhomocysteinaemia. *Eur J Vasc Endovasc Surg* 1997; **14**: 506–508.
130 Wassef M, Baxter BT, Chisholm RL *et al*. Pathogenesis of abdominal aortic aneurysms: A multidisciplinary research program supported by the National Heart, Lung, and Blood Institute. *J Vasc Surg* 2001; **34**: 730–738.

Accepted 25 April 2002